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(54) Title: DENDRITIC CELLS ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

(57) Abstract: The present invention provides novel methods for immunotherapy. The invention provides immune cells and methods to generate them, with the capacity to at least in part reduce an immune response in a host. In one aspect the invention provides a method for generating a dendritic cell with the capacity to tolerise a T cell for antigen said T cell was specific for, comprising culturing peripheral blood monocytes from an individual to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said activated dendritic cell with said antigen T cell was specific for.

Title: Dendritic cell activated in the presence of glucocorticoid hormones are capable of suppressing antigen-specific T cell responses.

The invention relates to the field of medicine. More in particular the invention relates to the field of immunotherapy.

Background of the invention

The remarkable immunostimulatory properties of DC reside in their ability to transport antigens from peripheral tissues to lymphoid organs where they present these antigens to T cells in an optimal costimulatory context (1). To achieve this complex sequence of events, DC exist in different functional stages. Immature DC behave as sentinels in peripheral tissues where they efficiently capture antigens. Upon pathogen invasion, induction of protective T cell responses requires the activation of immature DC into mature immunostimulatory cells. DC activation is triggered in inflamed tissues by cytokines such as IL-1 and TNF-a and by bacterial components such as LPS (2, 3). Activated DC migrate to T cell areas in the lymph nodes while upregulating their costimulatory capacities and optimizing their antigen presenting functions. Upon interaction with antigen-specific T cells, DC activation is further completed through engagement of the receptor-ligand (L) pair CD40-CD40L, leading to the production of IL-12 (4, 5, 6), a key cytokine for T helper (Th) type 1 and cytotoxic T lymphocyte (CTL) priming (7).

APC activation through CD40-CD40L interactions represents an important immunoregulatory step for the establishment of protective T cell immunity against pathogens and tumors (8, 9, 10). This process also plays a key role in the onset of destructive T cell-mediated disorders such as auto-immune diseases, allograft rejection and graft versus host disease (11, 12, 13). The current treatment of these

disorders largely relies on the administration of glucocorticoids (GC), which exert potent anti-inflammatory and immunosuppressive effects. Because GC negatively interfere with many aspects of T cell activation such as IL-2-driven proliferation and inflammatory cytokine production (reviewed in 14), activated T cells have long been considered as the main targets for GC action. Several lines of evidence now suggest a role for DC in GC-induced immune suppression. Moser et al (15) found that GC prevented the spontaneous activation of murine DC thereby decreasing their T cell stimulatory potential. Kitajima et al (16) showed that GC could hamper the T cell-mediated activation of a murine DC line. Viera et al reported that human DC exposed to GC were poor producers of IL-12 upon LPS stimulation (17). These findings only concern loss of typical DC features and therefore favor a simple inhibitory role of GC on DC activation. A more complex immunoregulatory action on the DC system has not been considered.

The present invention resulted from a detailed analysis of the impact of GC on the CD40-mediated activation of monocyte-derived DC. These DC develop after culture with GM-CSF and IL-4 (2, 18) or after transmigration through endothelial cells (19) and are known to mature into the most potent human Th1-type-inducing APC upon CD40 ligation (5, 20). Moreover, these APC can easily be generated in large numbers and are thereby the cells of choice for DC-based modulation of T cell immunity (21, 22). In contrast to previous studies, the present invention shows that GC such as dexamethasone (DEX) do not merely prohibit DC activation, but that it converts CD40 ligation on human monocyte-derived DC is transformed into an alternative activation pathway. DEX profoundly affect the CD40-dependent maturation of human monocyte-derived DC, not only by preventing the upregulation of costimulatory, adhesion and MHC surface molecules, but also by causing these cells to secrete the anti-inflammatory mediator IL-10 instead of the Th1 stimulatory cytokine IL-12. In agreement with

these phenotypic and functional changes, DC triggered through CD40 in the presence of DEX are poor stimulators of Th1-type responses. Most importantly, the present invention shows that such DC are able to induce a state of hyporesponsiveness in Th1 cells, indicating that these cells are capable of active suppression of Th1-type immunity.

As already mentioned above, the impact of GC on DC has been the subject of several previous studies by others. However, in contrast with the present invention, these studies only highlighted inhibitory effects of GC on the DC system. DEX was found to block the upregulation of CD80, CD86 and MHC class II molecules upon activation of murine spleen DC (15, 16), whereas very recently DEX was demonstrated to also prevent the differentiation of DC from monocyte precursors (28). In these studies, the inability of DC to acquire high expression of costimulatory and MHC molecules was accompanied with a decrease in their T cell stimulatory potential, but the effect of GC on IL-12 production was not investigated. On the other hand, Viera et al found that the effect of GC on LPS-induced DC activation consisted in a 4-fold reduction of IL-12p70 synthesis (17). This partial effect on IL-12 secretion contrasts with the complete suppression of IL-12p70 production which is subject of the present invention, and can be explained by the fact that their GC-treated immature DC were extensively washed prior to LPS stimulation. We indeed found that upon removal of GC, the effects of these drugs on immature DC were rapidly reversible. The continuous presence of GC during CD40 triggering of DC was clearly preferred in order to stably and completely modulate DC activation (data not shown). Taken together, previous findings indicated that the impact of GC on the DC system should be merely interpreted as an inhibitory event. Importantly, the present invention clearly demonstrates that GC such as DEX do not simply suppress DC activation but rather redirect this process towards a distinct functional program.

DC activation through engagement of CD40-CD40L is a key stimulatory event for the generation of effective Th1 and CD4-dependent CTL responses in vivo (10, 36, 37, 38). This pathway however is also involved in the development of unwanted T cell responses leading to autoimmune disease or organ-transplant rejection (11, 12, 13). Until now, treatment of patients suffering from such disorders largely relies on the systemic administration of GC hormones. This treatment does not only suppress pathogenic T cell responses but also induces a general state of immunosuppression and metabolic and endocrine side effects. The present invention demonstrates that activation of human monocyte-derived DC through CD40, in the presence of GC such as DEX, results in an IL-10-producing APC that is a poor stimulator for Th1-type responses and that can even confer hyporesponsiveness to Th1 cells. The present invention therefore indicates that such DC loaded with appropriate antigens can be exploited as a novel approach for specifically downregulating unwanted T cell responses in vivo.

The dentritic cells of the invention posses different capabilities than previously reported for dendritic cells. One can therefore consider these cells to be part of a class of cells distinct from the class formed by the "classical" dendritic cells. The dendritic cells of the invention can be used in a different way than the classical dendritic cells. The dendritic cells of the invention can for instance be used to suppress, at least in part, an undesired immune response in a host. In one aspect invention therefore provides a method for preparing a pharmaceutical composition for reducing an unwanted T cell response in a host, comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading

said activated dendritic cells with an antigen against which said T cell response is to be reduced. An unwanted T cell response can be any type of T cell response. For instance, but not limited to, a T cell response associated with an auto-immune disease or a transplantation disease such as a graft versus host disease or a host versus graft disease. A pharmaceutical composition of the invention typically comprises a dendritic cell of the invention suspended in a liquid suitable for preserving the function of said dendritic cell in said liquid and/or suitable for administration to a host. A host preferably is a human. Preferably said host is at risk of developing or is suffering from an auto-immune disease or allergy. Preferably, said host suffers from or is at risk of suffering from host versus graft disease and/or graft versus host disease. With the term at risk is meant that one expects that said host may develop said disease, for instance but not limited to a host receiving a transplant. Such a host is considered to at risk of developing host versus graft disease. An antigen typically is a peptide capable of binding to a major histocompatibility complex I and/or II molecule. Such peptides are known in the art and a person skilled in the art is capable of determining whether a given peptide comprises an antigen or not. An antigen may be derived from a naturally occurring protein. An antigen may also be a synthetic peptide or equivalent thereof, preferably with an amino-acid sequence equivalent to a peptide derived from a protein.

In another aspect the invention provides a pharmaceutical composition for reducing an unwanted T cell response in a host, said composition being obtained by culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said activated dendritic cells with an antigen against which said T cell response is to be reduced. In one embodiment a method is provided for reducing an unwanted T cell response in a

host, comprising administering a composition of the invention to said host.

The invention further provides method for reducing an unwanted T cell response in a host comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells and/or their precursors in the presence of a glucocorticoid hormone and loading said activated dendritic cells with an antigen against which said T cell response is to be reduced and administering said composition to said host.

In one embodiment of the invention said activation is done through a CD40 receptor. Activation of DC through triggering of the CD40 receptor can involve either incubation with a CD8-CD40L fusion protein, a trimeric from of CD40L consisting of CD40L-molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, or cells that express CD40L. Other signals that can be employed for the activation of DC as described in the present invention include lipopolysaccharide (LPS) and polyI/C.

In another aspect the invention provides a method for obtaining an dendritic cell capable of tolerising a T-cell for an antigen comprising providing said dendritic cell with a glucocorticoid hormone, activating said dendritic cell and providing said dendritic cell with said antigen. With the term tolerising is meant that said dendritic cell has an immunosuppressive effect on said T cell. A tolerised T cell will essentially not respond with cell division when exposed to a cell presenting an antigen said T cell would in the untolerised state respond to with cell division. A tolerised T cell will essentially not respond with killing a cell presenting an antigen said T cell would in the untolerised state respond to with cell kill.

In one embodiment said dendritic cell and/or a precursor thereof is provided with said glucocorticoid hormone <u>in vitro</u>. A T cell of the invention is preferably an antigen specific T cell, preferably a cytotoxic T cell or a Th cell.

In another aspect the invention provides an isolated dendritic cell capable of modifying the function of an antigen specific Th cell ,which would otherwise enhance a given immune response, resulting in a T cell that is capable of reducing this immune response. In one embodiment the invention provides a method for modifying an antigen specific T-cell comprising providing an dendritic cell according to the invention with said antigen and co-cultivating said T-cell and said dendritic cell. Preferably, said co-cultivating is performed in vitro. Said method may further comprise multiplying said functionally modified T-cell.

The invention also provides an isolated funtionally modified T-cell obtainable by a method according to the invention.

In another aspect the invention provides the use of a glucocorticoid hormone for obtaining an dendritic cell capable of functionally modifying a T-cell.

The invention also provides a pharmaceutical composition comprising an dendritic cell and/or a functionally modified T-cell according to the invention. The invention further provides the use of a dendritic cell and/or a functionally modified T-cell according the invention for the preparation of a medicament.

The invention also provides a method for the treatment of an individual suffering from or at risk of suffering from a disease associated with at least part of the immune system of said individual comprising providing said individual with an dendritic cell and/or a functionally modified T-cell according to the invention. Preferably, said dendritic cell and/or said functionally modified T-cell, or precursors thereof are derived from an HLA-matched donor. Preferably, said HLA-matched donor is said individual.

Method of treatments of the invention are preferably use for the treatment of an individual suffering from an auto-immune disease, an allergy, a graft versus host disease and/or a host versus graft disease.

Examples

Example 1

Impairment of CD40-CD40L-mediated phenotypic changes by DEX

We explored the impact of DEX on the phenotypic changes induced by CD40 ligation on immature monocyte-derived DC. In the absence of DEX, the fusion protein CD8-CD40L induced a strong upregulation of the costimulatory molecules CD80, CD86 and CD40, of the MHC class I and II molecules, of the adhesion markers CD54 and CD58 and of the DC maturation marker CD83 (Fig 1). In the presence of DEX, these CD8-CD40L-induced phenotypic changes were dramatically impaired: the upregulation of CD80, CD86, CD40, CD54, CD58 and of the MHC class I and II molecules was largely inhibited and CD83 was not expressed (Fig 1). Importantly, DEX-treated DC did not revert to a monocyte/macrophage stage as shown by the lack of expression of CD14 (Fig 1). Titration of DEX showed a complete inhibition of CD40-mediated phenotypic changes at 10^{-6} M and 10^{-7} M, a partial blockade at 10^{-8} M and no effect at 10^{-9} M and 10^{-10} (data not shown). In addition, DEX action was dependent on binding to the GC-receptor, since it was asbolished by simultaneous addition of the GC receptor antagonist RU486 (data not shown). In experiments performed with LPS or TNF-a as activation agents, similar results were obtained. However, the combination of DEX and TNF-alpha induced a massive cell death (viable cell recovery 5-10% of control cultures), a phenomenon that was not observed when DEX-treated DC were stimulated with LPS or through CD40 (viable cell recovery 60 to 100% of control cultures) (not shown).

We next analyzed whether activated DC could still be affected by DEX. DC incubated with CD8-CD40L for 48h and further exposed to DEX maintained a stable activated phenotype (Fig 2).

We conclude that DEX prevents the phenotypic changes induced by CD40 signals on immature DC and that already activated DC are resistant to DEX action.

Example 2

DEX does not interfere with the regulation of DC antigen uptake machinery

Unlike activated DC, immature DC efficiently internalize antigens through macropinocytosis and mannose receptor-mediated endocytosis (2, 3, 25, 26). We analyzed whether DEX could affect the DC antigen capture machinery and its downregulation following CD40 cross-linking. As shown in Fig 3, incorporation of FITC-BSA and FITC-mannosylated BSA by immature DC and by DEX-treated immature DC was comparable. Upon CD40 triggering, a similar decrease of FITC-BSA and FITC-mannosylated BSA uptake by both DEX-treated and untreated DC was observed (Fig 3). These results were the first to indicate to us that DEX does not block all aspects of DC activation, since it does not interfere with the down-regulation of the DC antigen capture machinery.

Example 3

DEX-treated CD40-triggered DC secrete IL-10 instead of IL-12

A key feature of CD40-triggered DC for initiating T cell immunity resides in their ability to produce the proinflammatory cytokine IL-12 (5, 6, 27). We investigated whether DEX affected IL-12 production by DC stimulated through CD40, and we explored the possibility that DEX could promote the secretion of the anti-inflammatory cytokine IL-10. As shown in Fig 4, CD40 triggering of DC strongly induced IL-12p40 and IL-12p70 secretion (up to 120ng/ml and 170pg/ml respectively) but only poorly stimulated the production of IL-10 (up to 68pg/ml). In contrast, CD40 triggering of DEX-treated DC resulted in a dramatically reduced IL-12p40 production (up to 100 fold) and in the complete suppression of IL-12p70 secretion, whereas IL-10 production was strongly

enhanced (up to 50 fold) (Fig 4). Immature DC and their DEX-treated counterparts failed to secrete detectable amounts of IL-12 and IL-10 (Fig 4). Therefore, CD40 ligation of DC in the presence of DEX triggers the secretion of high levels of the anti-inflammatory cytokine IL-10 instead of IL-12.

Example 4

DEX-treated CD40-triggered DC are capable of suppressing Th1-type immunity

The strikingly modified response of DC to CD40 ligation in the presence of DEX prompted us to compare the T cell stimulatory potential of these cells with that of their DEX-untreated counterparts. In an allogeneic MLR, CD40-triggered DC induced a strong proliferative T cell response whereas the addition of DEX prior to CD40 triggering reduced their T cell stimulatory capacity to that of immature DC (Fig 5). When tested for their ability to stimulate an hsp65-specific $CD4^+$ Th1 clone, CD40-triggered DC pulsed with the hsp65 protein or with the specific peptide epitope p3-13 were found to be potent inducers of both T cell proliferation and T-cell dependent IFN-g production (Fig 5). In contrast, in the presence of Ag-pulsed DEX-treated CD40-triggered DC, T cell proliferation and IFN-g production were significantly decreased ($p<0.001$ and $p<0.01$ respectively) (Fig 5). We next investigated whether DEX-treated CD40-triggered DC were simply poor stimulators of Th1 cells, or whether they could exert suppressive effects on these T cells. We therefore tested hsp65-specific T cells stimulated with p3-13-pulsed DEX-treated CD40-triggered DC for their capacity to respond to a second potent antigenic challenge. Fig 6 shows that preculturing T cells with CD40-triggered DC led to a strong T cell proliferation and IFN-gamma production upon second antigen-specific restimulation. In contrast, preculture with DEX-treated CD40-triggered DC resulted in a dramatically reduced proliferative and IFN-gamma production capacity of Th1 cells. Thus, CD40 triggering of DC in the presence of DEX

results in APC that are not merely poor inducers of T cell responses but that also induce a state of hyporesponsiveness in Th1 cells.

Example 5

DEX-treated activated DC suppress anti-transplant immunity in vivo

In a first attempt to test whether DEX-treated DC could suppress unwanted immunity, a skin transplant model was set up in mice. Skin transplants from C57Bl/6 mice, when grafted onto fully allogeneic Balb/c recipient mice, are known to be rapidly rejected as a result of a very strong allo-reactive anti-transplant T cell immune response. Experiments were performed to analyse whether pre-treatment of the recipient mice with DEX-treated DC resulted in suppression of this host versus graft response. We made use of an established murine DC cell line of C57Bl/6 origin, named D1, which exhibits a stable immature phenotype in vitro unless they receive an activating signal through agonistic anti-CD40 antibodies or LPS. This trigger results in fully matured DC that are highly capable of CTL priming in vitro as well as in vivo (39;40). Maturation of D1 involves strong upregulation of the cell surface expression of the costimulatory molecule CD86 (B7.2), the CD40-receptor and Class II MHC (fig. 7). Similar to our observations in human DC, maturation of D1 in the presence of DEX largely prevented the upregulation of these molecules at the cell surface (Fig. 7). This suggested that the DEX-treated D1 cells could be exploited to suppress T cell immunity in vivo. Balb/c mice were therefore injected with D1 cells that had been activated in the absence or presence of DEX. One week after immunisation, the allo-reactive response of splenocytes of these mice, as well as of control mice, was tested in vitro. Mice pre-treated with immature D1 cells that had been cultured in the presence or absence of DEX did not exhibit significantly altered Th1-type alloreactive immunity

as compared to control mice (Fig. 8). This is in accordance with our observations with human DC, which also showed that DEX-treated immature DC did not exhibit different properties than normal immature DC. As expected, mice immunised with fully matured D1 showed enhanced responsiveness. Importantly, mice pre-treated with D1 activated in the presence of DEX displayed greatly decreased Th1-type allo-reactive immunity (fig. 8).

It was subsequently tested whether the reduction of allo-responses in the latter group of mice, as measured in vitro, would reflect decreased allo-reactive immunity in vivo. Groups of Balb/c mice were immunized with D1 cells matured in the absence or presence of DEX. Skin transplants of C57Bl/6 origin were grafted onto these mice one week later, and graft survival was monitored. The grafts were rapidly rejected in control mice and mice immunized with normally matured D1 cells (Fig. 9). Rejection was not enhanced in the latter group, despite the stronger allo-reactive responses in these mice (Fig. 8). Most likely, the response in the control mice is already sufficient for maximal rejection. Importantly, graft rejection is significantly ($p < 0.05$) delayed in mice pre-treated with D1 matured in the presence of DEX (fig. 9). This outcome corresponds with the greatly reduced allo-reactivity in these mice as determined in vitro (Fig. 8).

In conclusion, the skin grafting experiments in mice demonstrate that DC matured in the presence of DEX can indeed be employed to suppress unwanted immunity such as against transplants. Although in the experiments shown the transplants are eventually rejected, it should be noted that donor and recipient displayed large differences with respect to transplantation antigens. In humans, donors and recipients are matched as closely as possible for these antigens. Murine experiments exploiting better matched donor and recipient strains are underway. The data in Fig. 8 and 9 show that pre-treatment with DEX-treated mature DC will result in much more profound effects in a this setting.

Materials and Methods

Generation of DC

Immature DC were generated from peripheral blood monocyte precursors. Human PBMC from healthy donors, isolated through Ficoll-Hypaque density centrifugation were plated at $1.5x10^7$ per well in 6-well plates (Costar Corp., Cambridge, MA) in RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 2mM glutamine, 100UI/ml penicillin and 10% FCS. After 2 h at 37^0C, the non adherent cells were removed and the adherent cells were cultured in medium containing 500U/ml IL-4 (Pepro Tech Inc. Rocky Hill, NJ) and 800U/ml GM-CSF (kindly provided by Dr S. Osanto, LUMC, Leiden, NL) for a total of 7 days.

Activation of immature DC with a CD8-CD40L fusion protein

Activation of DC though CD40 was performed with a fusion protein made of the extracellular domain of human CD40L and of the murine CD8a chain (CD8-CD40L). The CD8-CD40L cDNA described by Garrone et al (23) was transferred into an eukaryotic expression vector containing the hygromycin resistance gene, and used for the generation of stably transfected Chinese Hamster Ovary (CHO) cells. Culture supernatants containing the CD8-CD40L fusion protein were concentrated with a pressurized stirred cell system (Amicon, Inc., Beverly, MA), checked for binding to CD40 and tested for optimal DC activation conditions (not shown). DC were incubated at 5×10^5/ml/well in a 24-well plate (Costar Corp., Cambridge, MA) and activated in the presence of 1/10 CD8-CD40L supernatant. Cells and supernatants were analyzed after 48 h. Of note, control supernatants obtained from untransfected CHO cells or from CHO cells transfected with

the CD8a cDNA lacked DC activating functions and were similar to culture medium.

DEX and RU486 treatment of DC

Seven days immature DC were treated with 10^{-6}M DEX (Sigma, St Louis, MO) in the presence of GM-CSF and IL-4 or GM-CSF alone. After 24 h, DC were analyzed or were further stimulated via CD40 by adding the CD8-CD40L fusion protein to the cultures as described above. In some experiments, the glucocorticoid receptor antagonist RU485 (Roussel-UCLAF, Romainville, France) was used at 10mM final concentration, alone or in combination with DEX.

Analysis of DC surface phenotype by flow cyttometry

Cells were stained on ice with FITC or PE-conjugated mouse monoclonal antibodies (MoAb) for 30 min in PBS 1% FCS and were analyzed on a FACScan® (Becton Dickinson, San Jose, CA). The following MoAb were used: FITC-anti-CD80 (BB1), PE-anti-CD86 (FUN-1), FITC-anti-CD40 (5C3), PE-anti-CD54 (HA 58) and PE-anti-CD58 (1C3) (Pharmingen, San Diego, CA), PE-anti-CD14 (L243) and PE-anti-HLA-DR (Mf-P9) (Becton Dickinson), PE-anti-CD83 (HB15A) (Immunotech, Marseille, France) and PE-anti-HLA class I (Tu 149) (Caltag Laboratories, Burlingame, CA).

Antigen uptake experiments

DC were resuspended in medium buffered with 25mM Hepes. FITC-BSA and FITC-mannosylated BSA (both from Sigma) were added at 1mg/ml final concentration and the cells were incubated at 37^0C, or at 0^0C to determine background uptake. After 1 h, DC were washed extensively with iced-cold PBS and analyzed by FACS® using propidium iodide to eliminate dead cells.

Cytokine detection by ELISA

Culture supernatants were analyzed in serial twofold dilutions in duplicate. IL-12p70 was detected using a solid phase sandwich ELISA kit (Diaclone Research, Besancon, France) (sensitivity 3pg/ml). For IL-12p40 and IFN-g detection, capture MoAb and polyclonal biotinylated detection Ab were obtained from Peter van de Meijde (BPRC, Rijswijk, NL) (sensitivity 10pg/ml). IL-10 was detected using the Pelikine compact human IL-10 ELISA kit (CLB, Amsterdam, NL) (sensitivity 3pg/ml).

Allogeneic mixed lymphocyte reaction (MLR)

Non adherent allogeneic adult PBMC from an unrelated individual were cultured in 96-well flat-bottom plates (Costar Corp., Cambridge, MA) at a density of 1.5×10^5/well with various numbers of g-irradiated (3,000 rads) DC, in triplicates. Proliferation was assessed on day 5 by [^{3}H]thymidine uptake (0.5mCi/well, specific activity 5Ci/mMol, Amersham Life Science, Buckinghamshire, UK) during a 16 h pulse.

Th1 stimulation assays

The Mycobacterium tuberculosis and M. leprae hsp65-specific, HLA-DR3-restricted CD4+ Th1 clone Rp15 1-1 used in this study recognizes an hsp65 determinant corresponding to peptide residues 3 to 13 (p3-13) (24). HLA-DR-matched DEX-treated immature DC and their DEX-untreated counterparts were pulsed with 10mg/ml of p3-13 or with 10mg/ml of hsp65 for 2 h, washed extensively and stimulated through CD40 as described above. For Ag-pulsed DEX-treated immature DC, CD40 triggering was performed in the presence of DEX. Hsp65 specific T cells (10^4) were cultured with different numbers of g-irradiated (3,000 rads) DC in 96-well flat-bottom plates (Costar Corp.) in triplicates for 3 days. [^{3}H]thymidine incorporation was measured on day 3 after a 16 h pulse. Before the addition of

[^{3}H]thymidine, 50ml of supernatants were collected from each well and supernatants from triplicate wells were pooled to measure IFN-g production. To test hsp65-specific T cells responsiveness to a second potent antigenic challenge, 10^4 T cells were first cultured for 48h with 5 x 10^3 peptide-pulsed DC prepared as above, then harvested and allowed to rest in medium containing 5U/ml IL-2. Three days later, 10^4 viable T cells were restimulated with 5 x 10^3 peptide-pulsed DC generated from the same donor as used for the first culture and tested for their ability to proliferate and to produce IFN-g as previously described.

Statistical analysis

Covariance analysis was used to compare T cell proliferation and IFN-g production as a function of DC number, between DEX-treated CD40-triggered DC and DEX-untreated CD40-triggered DC (Fig. 5).

Figure legends

Fig. 1 Pretreatment with DEX inhibits the phenotypic changes induced by CD40 ligation.
Seven days immature DC were cultured for 24h in the absence or the presence of 10^{-6}M DEX and activated via CD40 with the CD8-CD40L fusion protein for 48h. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicated cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 4. Data are representative of 4 independent experiments.

Fig. 2 DC triggered through CD40 maintain an activated phenotype upon a subsequent DEX exposure.
Immature DC were activated with the CD8-CD40L fusion protein. DEX (10^{-6}M) or medium control were added 48h later and cells were analyzed after 2 additional days of culture. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicated cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 5. Data are representative of 2 independent experiments.

Fig. 3 Pretreatment with DEX does not affect the regulation of DC antigen uptake machinery.
Immature DC were incubated in the absence or the presence of 10^{-6}M DEX for 24h and further activated or not via CD40 with the CD8-CD40L fusion protein for 48h. Cells were pulsed for 1h with medium containing either 1mg/ml FITC-BSA or 1mg/ml FITC-mannosylated BSA. Empty histograms show the background

autofluorescence, Grey-filled histograms show the background uptake at 0^0C and black-filled histograms show the specific uptake at 37^0C. Data are representative of 3 independent experiments.

Fig. 4 Pretreatment with DEX alters the cytokine secretion profile of CD40-triggered DC.
DEX-exposed or control immature DC were left in culture without further treatment or stimulated with the CD8-CD40L fusion protein. Culture supernatants were harvested 48h later and IL-10, IL-12p40 and IL-12p70 secretion were analyzed by specific ELISA. Data are representative from 6 independent experiments.

Fig. 5 Pretreatment with DEX impairs the T cell stimulatory capacities of DC activated via CD40 and leads to a state of hyporesponsiveness of Th1 cells.
Allogeneic MLR: non adherent allogeneic PBMC were cultured with different numbers of CD40-triggered DC, DEX-treated CD40-triggered DC or immature DC. The proliferative response was measured on day 5.
Th1 stimulation assays: Hsp65-specific T cells were cultured with different numbers of HLA-DR matched CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the hsp65 protein or with the specific p3-13 peptide epitope. The proliferative response and the T cell dependent IFN-g production were analyzed on day 3. Data are representative of 4 independent experiments.

Fig. 6 DEX-treated DC triggered through CD40 induce a state of hyporesponsiveness in Th1 cells. Hsp65-specific T cells precultured with CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the p3-13 peptide epitope were harvested after 48h, allowed to rest in the presence of 5U/ml IL2 for 3 days, and restimulated with p3-13-pulsed DC. The

proliferative response and IFN-g production were measured on day 3. Similar results were obtained in 2 independent experiments.

Fig. 7 D1 cells were left untreated or cultured for two days in the presence of LPS (1 ug/ml) and/or DEX (10-6 M). Surface expression of CD86 (B7.2), Class II MHC and CD40 was monitored by staining with the appropriate FITC-labeled Ab. and subsequent FACS-analysis. Similar results were obtained for D1 cells matured in the presence of murine CD40L (not shown).

Fig. 8 Balb/c mice were immunised with 10-6 D1 cells that were prepared as indicated. One week later, splenocytes were isolated and tested for their response against C57Bl/6 splenocytes in a mixed lymphocyte culture. After 24 h, culture supernatants were tested for IFNg content in a specific ELISA assay.

Fig. 9 Balb/c mice (5 per group) were immunised as indicated. One week later, skin transplants of C57Bl/6 origin were grafted and graft survival was monitored.

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CLAIMS

1. A method for preparing a pharmaceutical composition for reducing an unwanted T cell response in a host, comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced.

2. A pharmaceutical composition for reducing an unwanted T cell response in a host, said composition being obtained by culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced.

3. A method for reducing an unwanted T cell response in a host, comprising administering a composition of claim 2 to said host.

4. A method for reducing an unwanted T cell response in a host comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced and administering said composition to said host.

5. A method according to claim 1,3 or 4 whereby said activation is done through a CD40 receptor.

6. A method according to claim 5 whereby said activation involves incubation of the dendritic cells with either CD8-CD40L fusion protein, a trimeric from of CD40L consisting of CD40L-molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, or cells that express CD40L.

7. A method according to claim 5 whereby said activation involves incubation of the dendritic cells with lipopolysaccharide (LPS) or polyI/C.

8. A method according to claim 1, 3-7 whereby said dendritic cells are infected with one or more recombinant viruses encoding the antigen(s) of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

9. A method according to claim 1, 3-8 whereby said dendritic cells are incubated with one or more recombinant proteins or large (> 20 amino acids) synthetic peptides representing the antigen(s) of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

10. A method according to claim 1,3 or 9 whereby said dendritic cells are incubated with cells or cell homogenate containing the antigen(s) of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

11. A method according to claim 1, 3-10 whereby said dendritic cells are loaded with synthetic peptides representing the antigen(s) of interest after activating said dendritic cells in the presence of a glucocorticoid hormone.

12. A method according to claim 1, 3-11 whereby said dendritic cells, after activation in the presence of a glucocorticoid hormone, secrete interleukin-10.

13. A method for obtaining a dendritic cell capable of tolerising a T-cell for an antigen comprising providing said dendritic cell with a glucocorticoid hormone, activating said dendritic cell and providing said dendritic cell with said antigen.

14. A method according to anyone of claims 1, 3-13, wherein said dendritic cell and/or a precursor thereof is provided with said glucocorticoid hormone in vitro.

15. A method according to anyone of claims 1, 3-14, wherein said T-cell is a T-helper cell.

16. An isolated dendritic cell prepared according to anyone of claims 1, 3-15 capable of functionally modifying an antigen-specific T-cell with respect to the response to said antigen.

17. A method for functionally modifying an antigen-specific T-cell comprising providing an dendritic cell according to claim 16 with said antigen and co-cultivating said T-cell and said dendritic cell.

18. A method according to claim 17, wherein said co-cultivating is performed in vitro.

19. A method according to claim 17 or claim 18, further comprising multiplying said functionally modified T-cell.

20. An isolated functionally modified T-cell obtainable by a method according to anyone of claims 17-19 that is capable, upon administration to the host, of reducing an unwanted immune response.

21. Use of a glucocorticoid hormone for obtaining an dendritic cell capable of functionally modifying a T-cell.

22. A pharmaceutical composition comprising an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20.

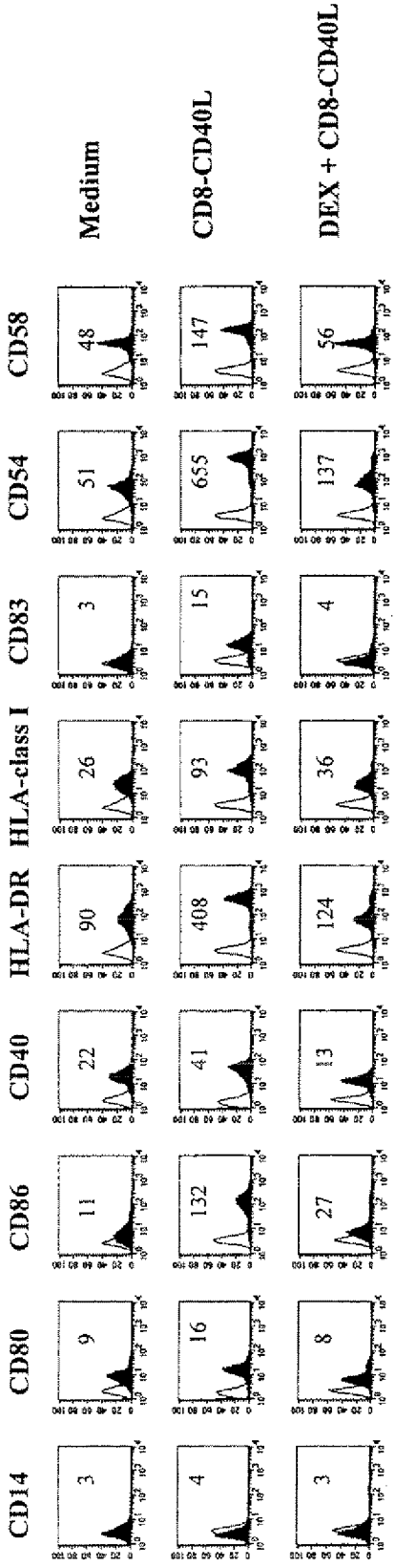
23. Use of an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20 for the preparation of a medicament.

24. A method for the treatment of an individual suffering from or at risk of suffering from a disease associated with at least part of the immune system of said individual comprising providing said individual with an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20.

25. A method according to claim 24, wherein said dendritic cell and or said T-cell is derived from an HLA-matched donor.

26. A method according to claim 24 or claim 25, wherein said dendritic cell and or said T-cell is derived from said individual.

27. Use of an dendritic cell according to claim 16 in a treatment for an individual suffering from an auto-immune disease, allergy, a graft versus host disease and/or a host versus graft disease.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9
11
22
90
26
3
51
48
4
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13
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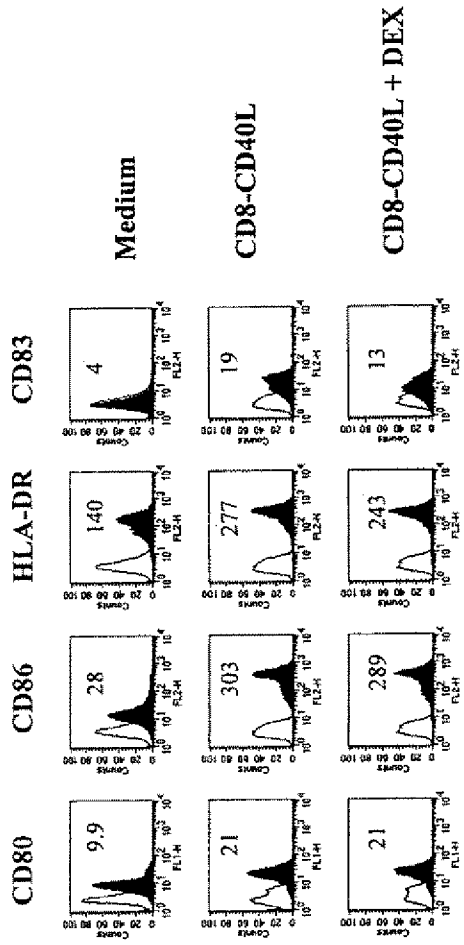
Fig. 1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21

Fig. 2

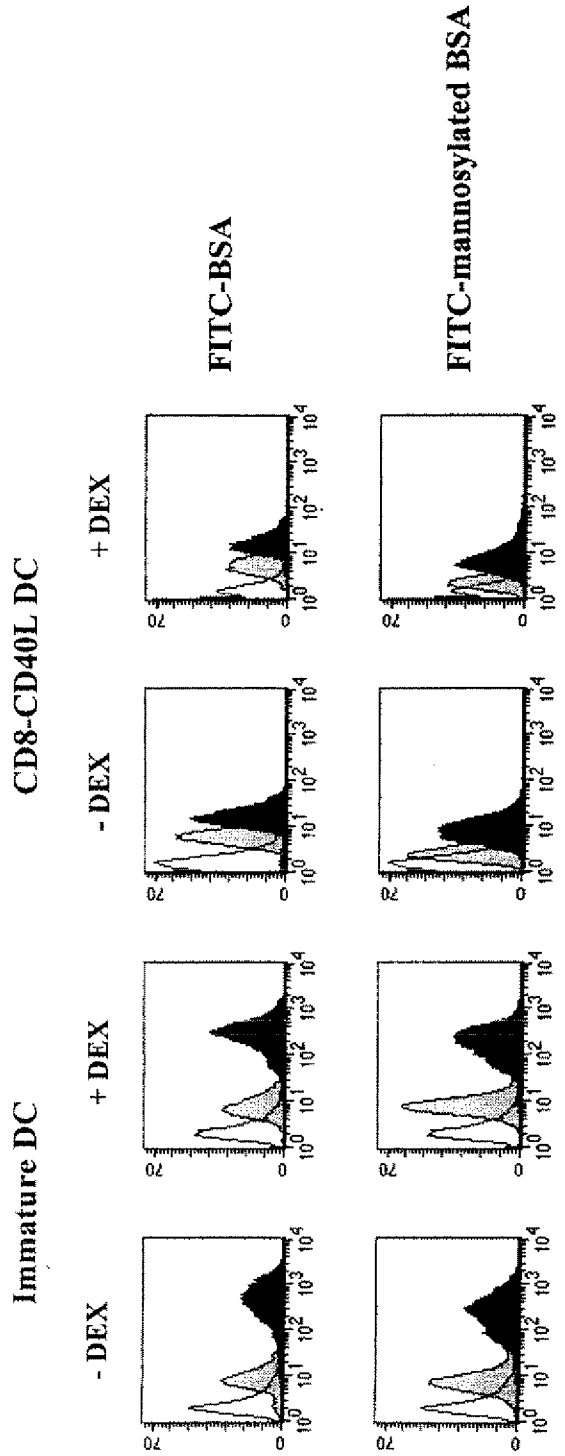
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Fig. 3

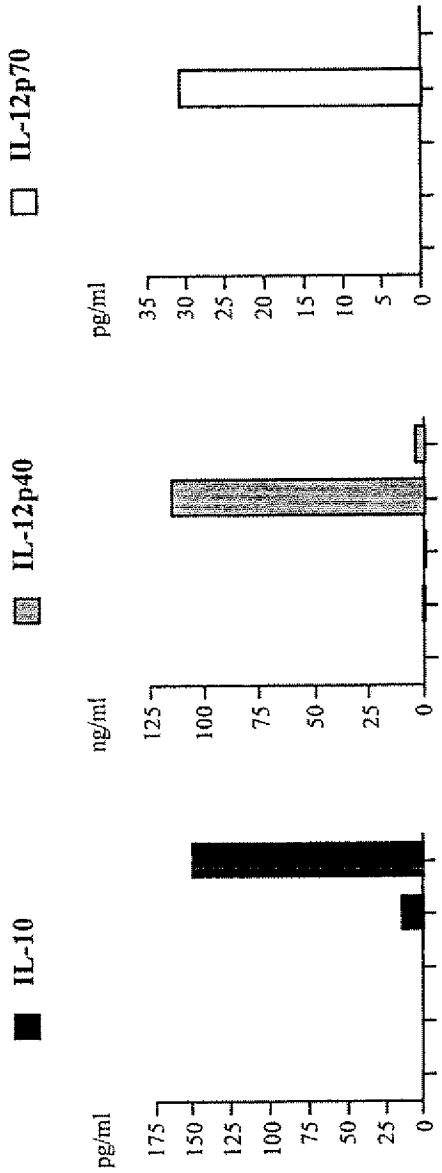
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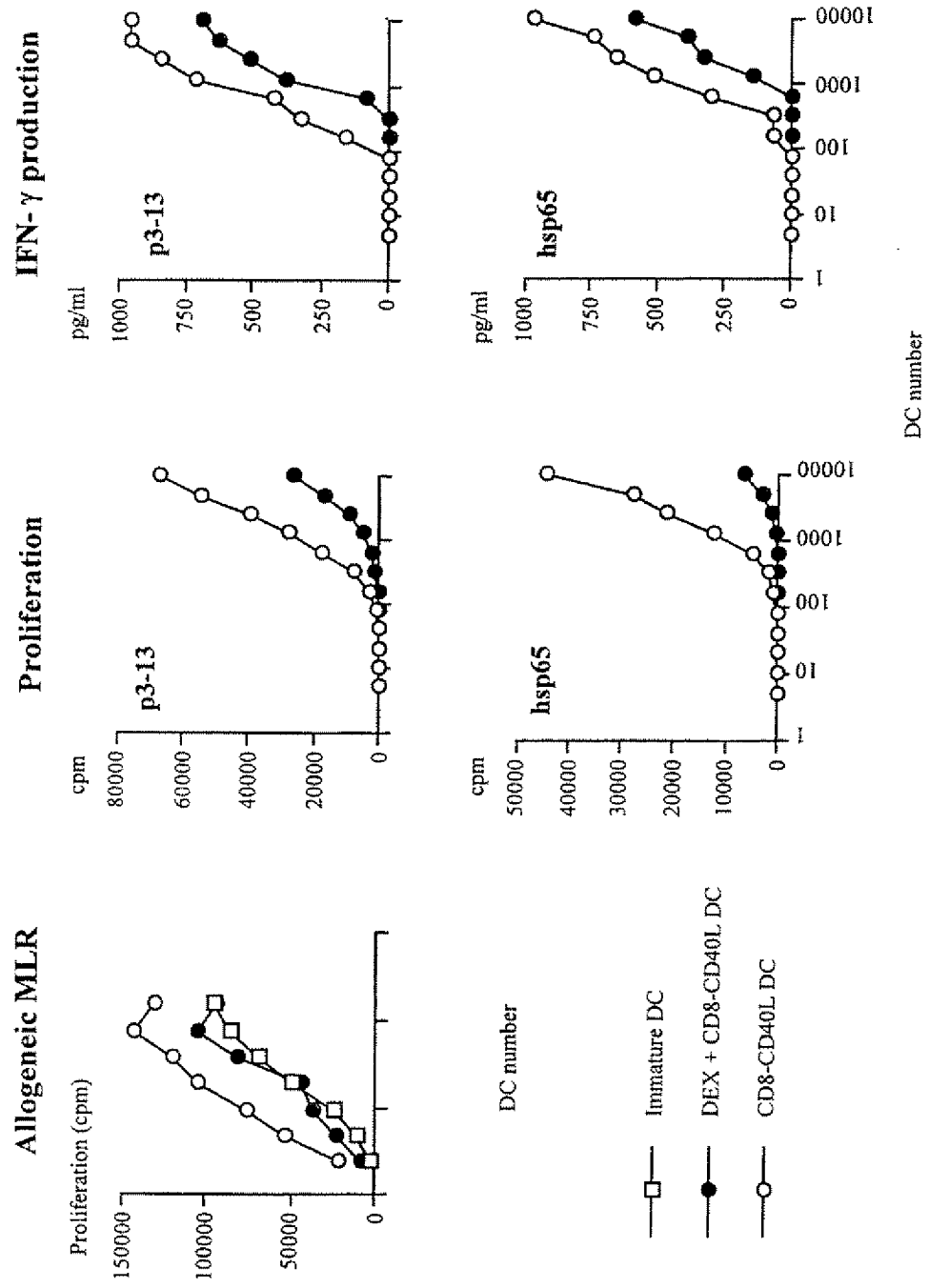
Fig. 4


Allogeneic MLR
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50000
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Proliferation
cpm
80000
60000
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cpm
50000
40000
30000
20000
10000
0
hsp65
IFN-γ production
pg/ml
1000
750
500
250
0
p3-13
pg/ml
1000
750
500
250
0
hsp65
1
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100
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DC number
DC number
Immature DC
DEX + CD8-CD40L DC
CD8-CD40L DC

Fig. 5

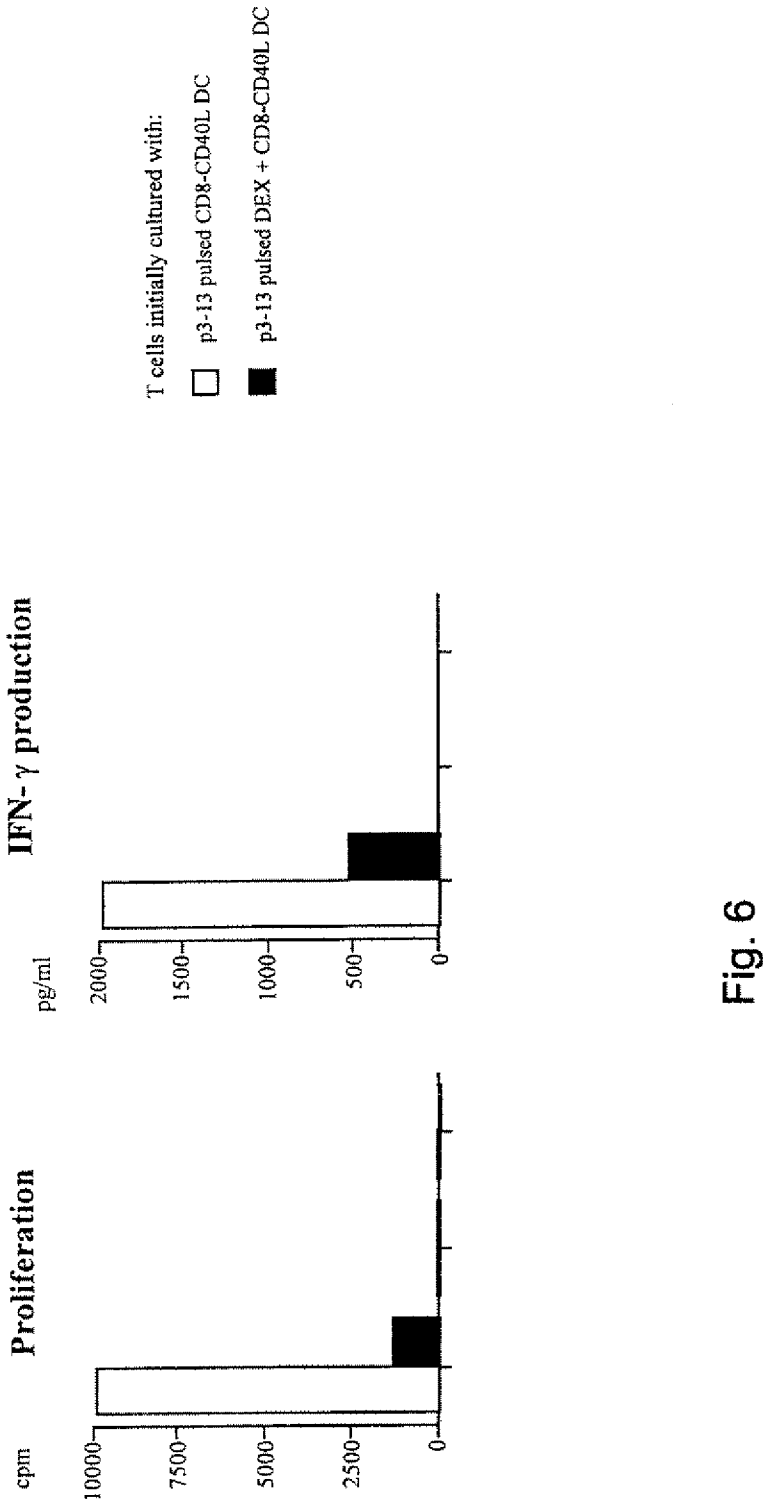

T cells initially cultured with:
p3-13 pulsed CD8-CD40L DC
p3-13 pulsed DEX + CD8-CD40L DC
IFN-γ production
pg/ml
2000
1500
1000
500
0
Proliferation
cpm
10000
7500
5000
2500
0

Fig. 6

Fig. 7. DEX modulates LPS-induced maturation of murine DC

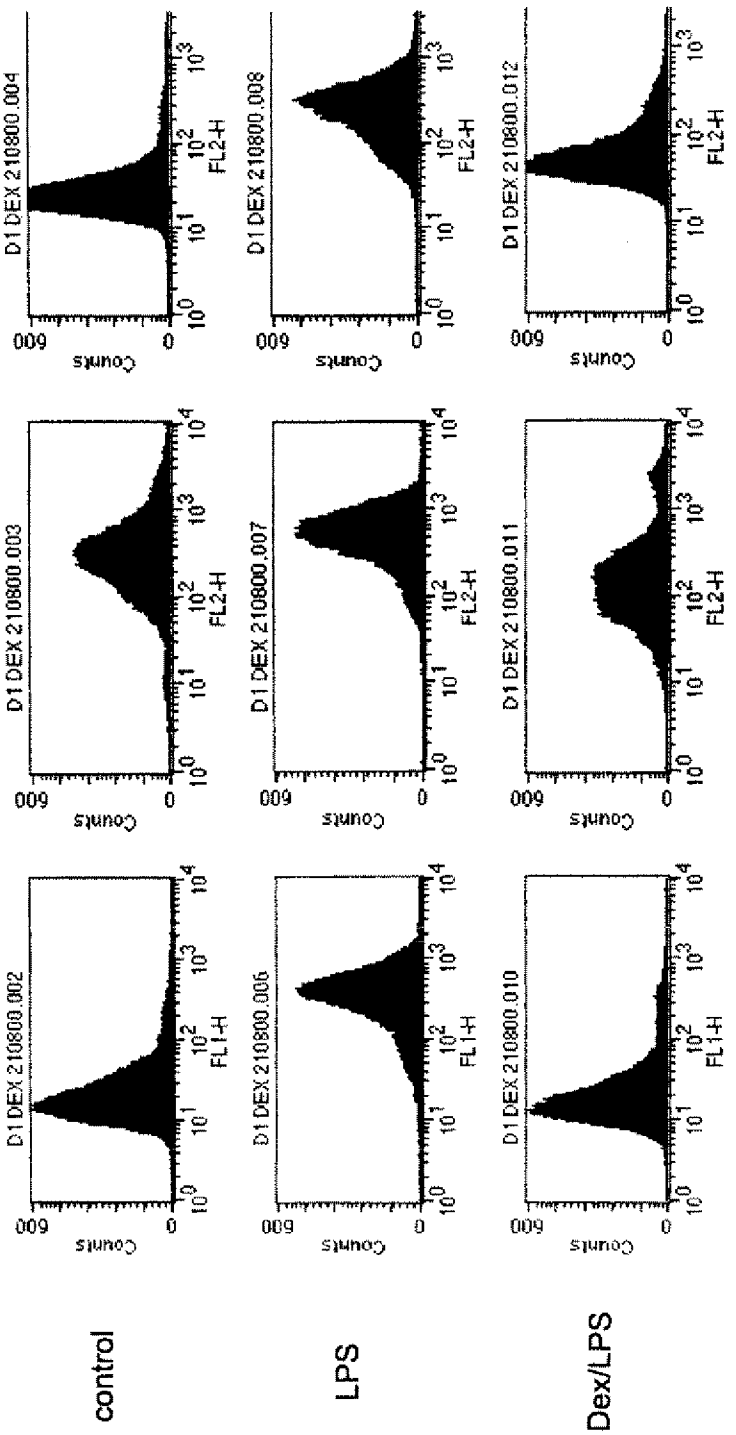


Figure 8. Decreased allo-reactivity after pre-treatment with DEX-DC

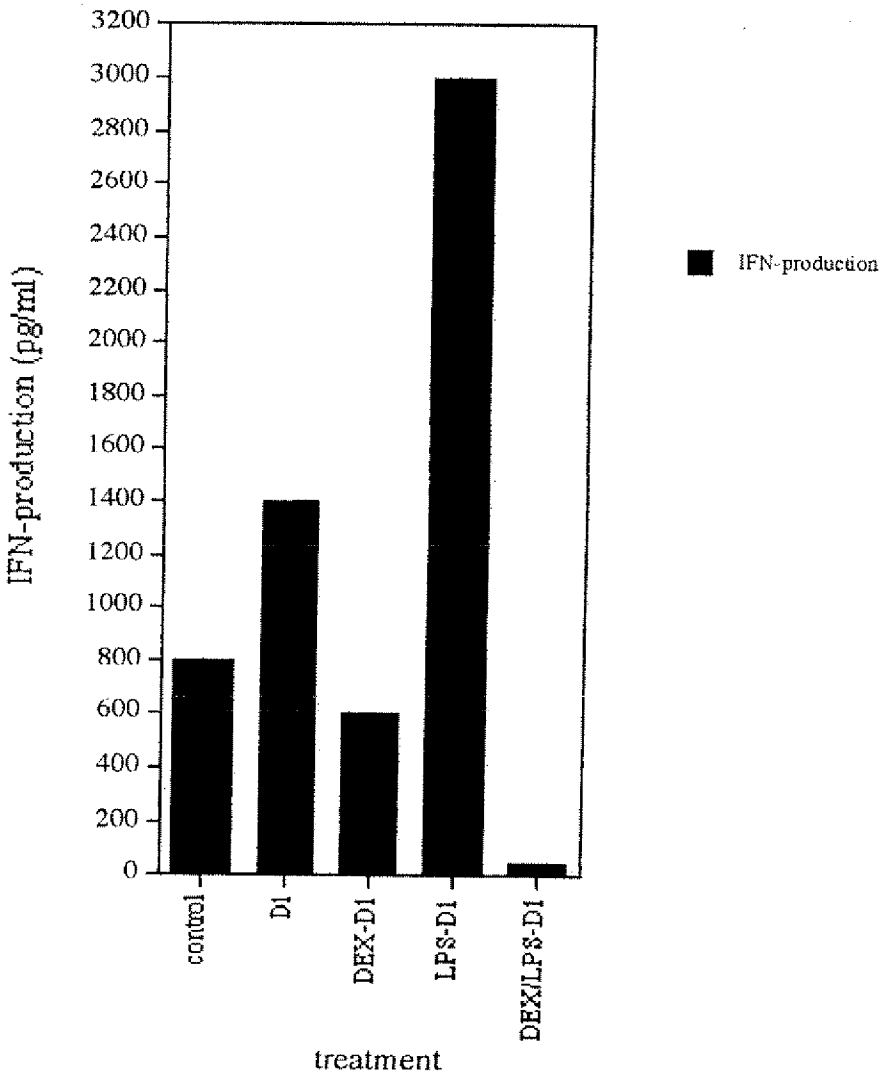
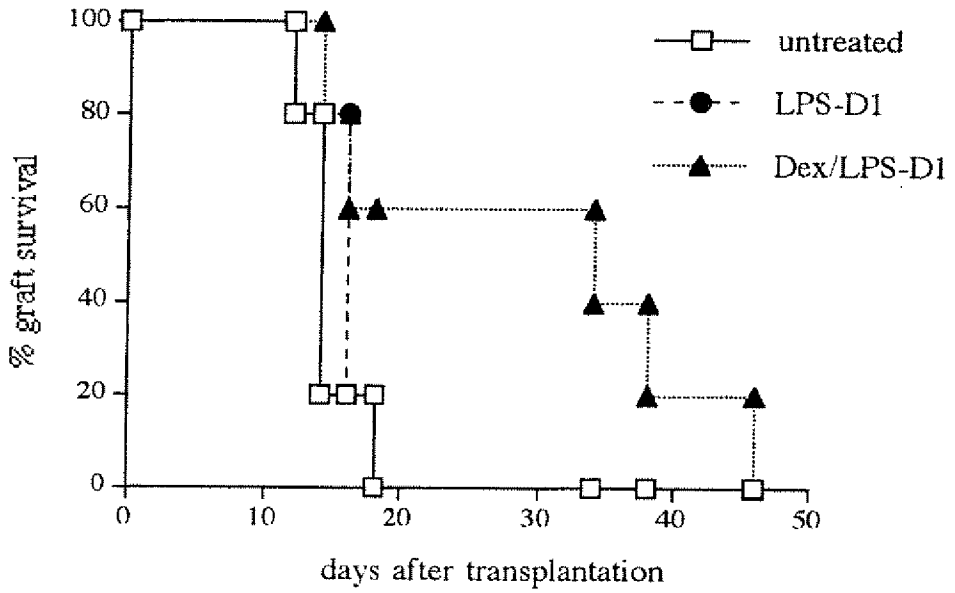


Figure 9. Prolonged graft survival after pre-treatment with DEX-DC



INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00712

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/00 A61K35/14 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VIEIRA P L ET AL: "Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential." JOURNAL OF IMMUNOLOGY, vol. 161, no. 10, 15 November 1998 (1998-11-15), pages 5245-5251, XP000973668 cited in the application	1-7, 9-16, 22-27
Y	the whole document	8-12, 14-16, 22-27

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2001	02.03.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bilang, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ir. ational Application No
PCT/NL 00/00712

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	LU L ET AL: "Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients" GENE THERAPY,MACMILLAN PRESS LTD., BASINGSTOKE,GB, vol. 6, no. 4, 1999, pages 554-563, XP000946114 ISSN: 0969-7128 the whole document	8-12, 14-16, 22-27
A	PIEMONTI L ET AL: "Glucocorticoids affect human dendritic cell differentiation and maturation." JOURNAL OF IMMUNOLOGY, vol. 162, no. 11, 1 June 1999 (1999-06-01), pages 6473-6481, XP002159692 cited in the application the whole document	1-27
A	GAO J X ET AL: "CD40-deficient dendritic cells producing interleukin-10, but not interleukin-12, induce T-cell hyporesponsiveness in vitro and prevent acute allograft rejection." IMMUNOLOGY, vol. 98, no. 2, October 1999 (1999-10), pages 159-170, XP000973593 the whole document	1-27
A	CHAKRABORTY ABALOKITA ET AL: "Stimulatory and inhibitory maturation of human macrophage-derived dendritic cells." PATHOBIOLOGY, vol. 67, no. 5-6, March 2000 (2000-03), pages 282-286, XP002159693 ISSN: 1015-2008 the whole document	1-27
P,X	REA D ET AL: "Glucocorticoids transform CD40-triggering of dendritic cells into an alternative activation pathway resulting in antigen-presenting cells that secrete IL-10." BLOOD , vol. 95, no. 10, 15 May 2000 (2000-05-15), pages 3162-3167, XP002159694 the whole document	1-27

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00712

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim(s) 3-12, 14, 16, 17, 19, 24, and 27 are directed to a diagnostic method practised on the human/animal body or treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.